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Chemical Composition of Lipophilic Extractives from *Eucalyptus globulus* Labill. Wood

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Keywords

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Summary

The composition of lipophilic extractives in the chloroform soluble fraction of the acetone extract from *Eucalyptus globulus* wood has been examined. The lipid extract was fractionated by solid-phase extraction on aminopropyl-phase cartridges into four different fractions of increasing polarity. The total lipid extract and the resulting fractions were analyzed by gas chromatography and gas chromatography-mass spectrometry, using high temperature capillary columns. The main compounds identified included sterols, sterol esters, fatty acids, steroid ketones, hydrocarbons and triglycerides. Minor compounds such as fatty alcohols, mono- and diglycerides, waxes and tocopherols were also identified among the lipids from *E. globulus* wood.

Introduction

Extractives from both softwoods and hardwoods cause production and environmental problems in the pulp and paper industry (Allen 1988). The accumulation of small amounts of wood extractives can result in blockages causing a shutdown of operations. These blockages are responsible for reduced levels of production, higher operating costs and an increased incidence of quality defects (Hillis and Sumimoto 1989). The viscous lumps that accumulate on equipment and the specks in pulp and paper, are referred to as pitch and they contain considerable amounts of wood lipophilic compounds (Mutton 1962). Lipophilic wood extractives are comprised mainly of fatty acids, resin acids, waxes, alcohols, terpenes, sterols, sterol esters and glycerides (Sjöström 1993) and the different classes of extractives have different chemical behavior during and after pulping. In neutral to acidic processing of the wood, the lipophilic extractives are difficult to remove, and resinous woods, are more of a problem in pitch control. However, in alkaline processing, such as kraft process, the total extractives content may not be as important as the composition of the extractives (Dunlop-Jones *et al.* 1991). During kraft pulping, the glycerol esters are completely saponified and the fatty and resin acids dissolved. However, sterols and some sterol esters and waxes, do not form soluble soaps under the alkaline conditions used in kraft pulping, and therefore, have a tendency to deposit and cause pitch problems (Swan 1967; Affleck and Ryan 1969; Leone and Breuil 1998). The higher concentration of these compounds in relation to the saponifiable extractives is the main cause for pitch problems in the kraft pulping of some hardwoods commonly used in the pulp and paper industry, such as aspen or eucalypt (Swan 1967; Allen 1988; Allen *et al.* 1991; Dunlop-Jones *et al.* 1991; Sitholé *et al.* 1992; Chen *et al.* 1995; Leone and Breuil 1998).

On the other hand, pitch problems are likely to become more severe with the introduction of more environmentally friendly bleaching processes that have substituted chlorine gas with other reagents such as chlorine dioxide (*elementary chlorine free*, ECF, bleaching) or hydrogen peroxide or ozone (*totally chlorine free*, TCF, bleaching). Likewise, the increasing reuse of white water and the trend towards complete closure of water circuits to meet environmental protection requirements are leading to an increase in pitch concentrations which would result in higher deposition.

Finding solutions to this problem begins with the identification of the extractives present in wood that may lead to pitch formation. There have been many studies concerning the composition of extractives from some softwoods and hardwoods commonly used in the pulp and paper industry (Hillis 1962; Fengel and Wegener 1984; Rowe 1989). However, little is known about the composition of extractives from *Eucalyptus* wood which is used extensively for paper pulp manufacture in South-Western Europe. Among the different *Eucalyptus* species, the wood of *E. globulus* is the most economically-important raw material for paper pulp production in Spain. Published studies on the chemical composition of extractives from *E. globulus* wood refer mainly to the more polar fraction containing phenols and polyphenols (Yazaki and Hillis 1976; Charrier *et al.* 1992; Conde *et al.* 1995). Studies referring to the composition of lipophilic extractives from *E. globulus* wood have been scarce and only the occurrence of several free and esterified fatty acids, hydrocarbons and sitosterol were reported (Swan and Åkerblom 1967).

In this paper, the chemical composition of lipophilic extractives from *E. globulus* wood, which are likely to be involved in pitch formation during manufacture of kraft pulp from eucalypt wood is reported. A solid-phase extrac-

tion and a chromatographic procedure developed for the analysis of wood extractives (Gutiérrez *et al*. 1998) were used in the present study. Short and medium-length high temperature capillary columns with thin films, which allow the elution and separation of high-molecular-mass lipids such as waxes, sterol esters and triglycerides (Wakeham and Frew 1982; Lusby *et al*. 1984; Evershed *et al*. 1989; Sitholé *et al*. 1992; Örså and Holmbom 1994) were used for the gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses. This work is part of a wider project aimed at the evaluation of the viability of biotechnological solutions for eliminating problematic lipids from both wood and pulp by treatment with fungi and enzymes, respectively, thus introducing environmentally-friendly procedures in pulp and paper manufacture. The detailed characterization of lipophilic extractives from *E. globulus* wood will greatly assist the development of these biotechnological approaches.

Materials and Methods

Samples

Wood samples were collected from *Eucalyptus globulus* Labill. grown in Pontevedra (North Spain). The trees were cut at an age of 12-14 years.

Extraction

Samples, previously debarked and ground to sawdust, were Soxhlet-extracted with acetone (Panreac, Barcelona, Spain) for 6 h. The lipophilic extractives were obtained by redissolving the dried acetone extract in chloroform (Merck, Darmstadt, Germany) and evaporated under nitrogen to dryness.

Solid-phase extraction (SPE) fractionation

The lipid extract from *E. globulus* wood was fractionated by a SPE procedure in aminopropyl-phase cartridges (500 mg) from Waters (Division of Millipore, Milford, MA, USA). All solvents used were supplied by Merck. The dried chloroform extract was taken up in a minimal volume (< 0.5 ml) of hexane-chloroform (4:1) and loaded into the cartridge column previously conditioned with hexane (4 ml). The cartridge was loaded and eluted by gravity. The column was first eluted with 8 ml of hexane and subsequently with 6 ml of hexane-chloroform (5:1), then with 10 ml of chloroform and finally with 10 ml of diethyl ether-acetic acid (98:2). Each isolated fraction was dried under nitrogen and weighted. The purity of each isolated fraction was confirmed by GC and GC-MS.

Saponification of sterol esters and waxes

All reagents used were supplied by Merck. The fraction containing sterol esters and waxes was hydrolyzed by refluxing it with a 0.5 M solution of potassium hydroxide in 90 % ethanol for 8 h. Long reflux times were necessary since sterols esters are hydrolyzed very slowly by most reagents and may not react completely (Christie 1982). The solution was extracted thoroughly with hexane, dried over anhydrous sodium sulfate and the non-saponifiable materials recovered on removal of the solvent in a rotary evaporator. The aqueous layer was acidified with 6 M hydrochloric acid and extracted with hexane. The free fatty acids were recovered after drying the extract over anhydrous sodium sulfate and removing the solvent in the usual way. Both the neutral and acidic fractions were analyzed by GC and GC-MS.

Gas chromatography

A Hewlett Packard HP 5890 gas chromatograph equipped with a split-splitless injector and a flame ionization detector (FID) was used (Hewlett Packard, Hoofddorp, Netherlands). The injector and the detector temperatures were set at 300 °C and 350 °C respectively. Triplicate samples (1 µl) were injected in the splitless mode. Helium was used as the carrier gas at a rate of 2 ml min^{-1}. The capillary column used was a high temperature, polyimide coated fused silica tubing DB5-HT (5 m × 0.25 mm I.D., 0.1 µm film thickness) from J&W Scientific (Folsom, CA, USA), specially processed for use at 400 °C. The oven was temperature-programmed from 100 °C (1 min) to 350 °C (3 min) at 15 °C min^{-1}. A mixture of standard compounds (palmitic acid, sitosterol, cholesteryl oleate and triheptadecanoin) supplied by Sigma Chemical Co. (St. Louis, MO, USA) was used to prepare a calibration curve for the quantitation of wood extractives with a concentration range between 0.1 and 1 mg ml^{-1}. The correlation coeficient was higher than 0.99 in all the cases. All peaks were quantified by peak area.

Gas chromatography-mass spectrometry

The GC-MS analyses were performed on a Varian Star 3400 gas chromatograph (Varian, Walnut Creek, CA, USA) with an ion trap detector (Varian Saturn 2000) using a high temperature capillary column (DB-5HT, 15 m × 0.25 mm I.D., 0.1 µm film thickness; J&W Scientific). Helium was used as the carrier gas. The samples (1 µl) were injected with an autoinjector (Varian 8200) directly onto the column using a SPI (Septum-equipped Programmable Injector) system. The temperature of the injector during the injection was 120 °C, and 0.1 min after the injection was programmed to 380 °C at a rate of 200 °C min^{-1} and hold 10 min. The oven was temperature-programmed from 120 °C (1 min) to 380 °C (5 min) at 10 °C min^{-1}. The temperatures of the ion trap and the transfer line were set at 200 °C and 300 °C respectively. Compounds were identified by computer comparison of the mass spectra with those in the Wiley and Nist libraries, by mass fragmentography and, when possible, by comparison with standard compounds.

Results and Discussion

The total acetone extract from *E. globulus* wood accounted for 1.52 %. The lipophilic - chloroform soluble- compounds represented 0.26 % while the rest, 1.26 %, corresponded to polar compounds non-soluble in chloroform. The lipid extract was subsequently fractionated by SPE in aminopropyl-phase cartridges into four major fractions of increasing polarity. The first fraction, eluted with hexane, was enriched in sterol esters, waxes and hydrocarbons. The second fraction, eluted with hexane-chloroform (5:1), contained triglycerides. The third fraction, eluted with chloro-

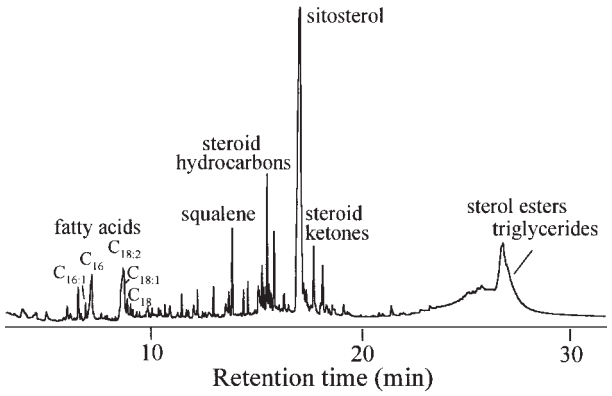

Fig. 1. Total Ion Chromatogram of lipophilic extractives from *E. globulus* wood. The identity of major compounds is shown on the chromatogram.

Table 1. Composition of lipids from *Eucalyptus globulus* wood

Compounds	Abundance (mg/100 g wood)
Hydrocarbons	**16.59 ± 0.90**
n-nonacosane	0.41
n-hentriacontane	1.15
n-tritriacontane	0.32
squalene	3.85
stigmasta-3,5-diene	8.20
other steroid hydrocarbons	2.66
Fatty acids	**27.69 ± 1.00**
n-tetradecanoic acid	0.70
n-pentadecanoic acid	0.56
9-hexadecenoic acid	1.03
n-hexadecanoic acid	8.60
n-heptadecanoic acid	0.22
9,12-octadecadienoic acid	7.55
9-octadecenoic acid	3.96
n-octadecanoic acid	2.50
n-eicosanoic acid	0.52
n-docosanoic acid	0.80
n-tetracosanoic acid	0.95
n-hexacosanoic acid	0.30
Fatty alcohols	**0.36 ± 0.05**
n-tetradecanol	0.03
n-hexadecanol	0.12
n-octadecanol	0.07
n-eicosanol	0.02
n-docosanol	0.03
n-tetracosanol	0.05
n-hexacosanol	0.04
Sterols	**64.50 ± 1.00**
cholesterol	1.00
campesterol	1.23
ergostanol	0.20
sitosterol	49.41
stigmastanol	5.93
fucosterol	2.50
cycloartenol	1.73
24-methylenecycloartenol	1.00
citrostadienol	1.50
Steroid ketones	**21.72 ± 0.50**
stigmastan-3-one	1.24
stigmasta-3,5-dien-7-one	8.90
stigmast-4-en-3-one	9.58
stigmasta-3,6-dione	2.00
Tocopherols	**2.50 ± 0.20**
α-tocopherol	2.50
Waxes (see Table 2)	**5.80 ± 0.50**
9-hexadecenoic acid esters	3.51
hexadecanoic acid esters	1.91
octadecanoic acid esters	0.38
Sterol esters (see Table 2)	**51.67 ± 1.70**
Monoglycerides	**1.58 ± 0.20**
Diglycerides	**1.70 ± 0.20**
Triglycerides	**13.21 ± 0.40**

form, contained sterols, fatty alcohols, diglycerides and monoglycerides. A final fraction enriched in free fatty acids was eluted with diethyl ether-acetic acid (98:2). The lipid extract and the SPE fractions were analyzed by GC and GC-MS. The chromatogram of the total lipid extract from *E. globulus* wood is shown in Figure 1 and the composition of all the compounds identified is listed in Table 1. Sterols, sterol esters, fatty acids, steroid ketones, hydrocarbons and triglycerides were major lipid groups present. Minor compounds such as fatty alcohols, mono- and diglycerides, waxes and tocopherols were also identified.

The most predominant compounds in the lipids of *E. globulus* wood were steroids, including sterols, sterol esters, steroid ketones and hydrocarbons. Sterols were the major compound class (64.50 mg/100 g wood), sitosterol being the main sterol present in the extract. Steroid ketones, accounting for 21.72 mg/100 g wood, were also another important components, being mainly constituted by stigmast-4-en-3-one and stigmasta-3,5-dien-7-one. Different steroid hydrocarbons (mono-, di- and triunsaturated) were also identified, stigmasta-3,5-diene being the most predominant.

Sterol esters were the second most important class of compounds present in *E. globulus* wood extractives, accounting for 51.67 mg/100 g wood. The complete identification of the individual sterol esters by GC-MS was not possible since they only show fragments arising from the sterol moiety by electron-impact MS and rarely give detectable molecular ions (Lusby *et al*. 1984; Evershed *et al*. 1989). By monitoring the ions corresponding to the different sterol moieties in the SPE fraction enriched in sterol esters, it was possible to identify series of sitosterol esters, with minor amounts of series of stigmastanol, cycloartenol and 24-methylenecycloartenol esters. However, it was not possible to individually characterize the fatty acyl moieties esterified to each sterol. For a more detailed characterization of the sterol ester composition, the SPE hexane fraction was subjected to saponification. The resulting neutral and acidic fractions were subsequently analyzed by GC and GC-MS (Fig. 2). The neutral fraction consisted of sterols and fatty alcohols arising from the saponification of sterol esters and waxes respectively, while the acidic fraction contained the fatty acids arising from both sterol esters and waxes. The compounds released after saponification are listed in Table 2. It can be observed that the distribution of esterified sterols is the same as that of free sterols, sitosterol being the predominant one. The esterified fatty acids, on the other hand, were identified in the range from C_{14} to C_{18}, with a predominance of linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acids. The distribution of the series of sitosterol and stigmastanol esters in the SPE hexane fraction is shown in Figure 3. It is interesting to note that both series have a similar distribution, and therefore it could be assumed that each sterol is esterified with the same distribution of fatty acids. Then, it could be possible to conclude that the major sterol ester present in *E. globulus* wood extractives is sitosteryl linoleate, that would represent more than 40 % of the total sterol esters, followed by sitosteryl oleate (14 %) and stigmastanyl linoleate (8 %).

Table 2. Composition (%) of sterols, fatty alcohols and fatty acids released after saponification of waxes and sterol esters

Compounds	%
Neutral fraction	
Sterols	
cholesterol	1.8
campesterol	0.7
ergostanol	0.1
sitosterol	66.8
stigmastanol	13.3
fucosterol	4.0
cycloartenol	3.7
24-methylenecycloartenol	2.3
citrostadienol	7.3
Fatty alcohols	
n-tetradecanol	8.5
iso-pentadecanol	2.5
n-pentadecanol	2.1
iso-hexadecanol	2.6
n-hexadecanol	9.0
iso-heptadecanol	4.3
n-heptadecanol	1.0
iso-octadecanol	4.5
n-octadecanol	11.4
iso-nonadecanol	2.6
n-nonadecanol	2.2
iso-eicosanol	5.9
n-eicosanol	9.9
iso-heneicosanol	7.3
n-heneicosanol	2.2
iso-docosanol	3.5
n-docosanol	6.6
iso-tricosanol	2.3
n-tricosanol	1.8
iso-tetracosanol	1.7
n-tetracosanol	4.8
iso-pentacosanol	1.4
n-pentacosanol	0.7
iso-hexacosanol	0.5
n-hexacosanol	0.7
Acidic fraction	
Fatty acids	
n-tetradecanoic acid	0.5
n-pentadecanoic acid	0.5
9-hexadecenoic acid	6.2
n-hexadecanoic acid	8.4
n-heptadecanoic acid	0.2
9,12-octadecadienoic acid	62.7
9-octadecenoic acid	20.6
n-octadecanoic acid	0.6
n-eicosanoic acid	0.3

Waxes represented only around 8 % of the total esters in the SPE hexane fraction. As revealed by GC-MS, the waxes were constituted exclusively by series of esters of palmitoleic ($C_{16:1}$), palmitic (C_{16}) and stearic (C_{18}) acids with different *normal*- and *iso*-fatty alcohols. The composition of the fatty alcohols released after saponification of waxes is listed in Table 2. It is interesting to note the identification of series of *normal*- and *iso*-fatty alcohols from C_{14} to C_{26}. While the *n*-alkanols have an even-over-odd predominance, the *iso*-alkanols have an odd-over-even predominance.

Free fatty acids and glycerides were also important constituents of *E. globulus* wood extractives. Free fatty acids accounted for 27.69 mg/100 g wood, and occurred in the range from C_{14} to C_{26}, the dominant component being palmitic acid (C_{16}), followed by linoleic ($C_{18:2}$), oleic ($C_{18:1}$) and stearic (C_{18}) acids. Triglycerides, accounting for 13.2 mg/100 g wood, were mainly constituted by tripalmitin (P_3). Mono- and diglycerides were also identified among the lipophilic extractives from *E. globulus* wood although in very minor amounts. The composition of these compounds is shown in Table 3.

Finally, other compounds identified among the *E. globulus* wood extractives were α-tocopherol and squalene, as well as minor amounts of series of *n*-alkanes (C_{29}, C_{31} and C_{33}) and fatty alcohols in the range from C_{14} to C_{26}, as reflected in Table 1.

Conclusions

The composition of lipophilic extractives from *E. globulus* wood has been studied. Sterols, sterol esters, fatty acids, steroid ketones, hydrocarbons and triglycerides were the major compounds identified. The identification of these compounds will lead to the design of effective biotechnological or physico-chemical solutions for the removal of the

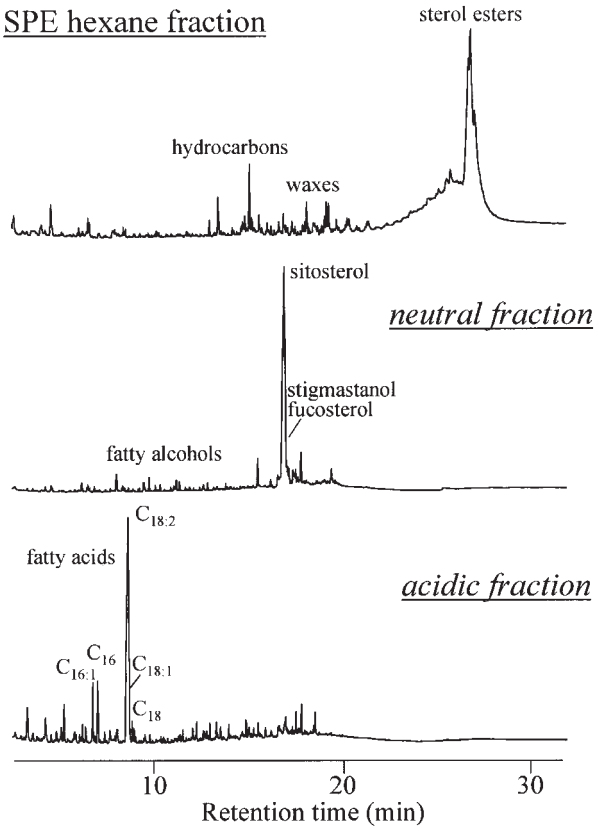

Fig. 2. Total Ion Chromatograms of the SPE hexane fraction, and the neutral and acidic fractions isolated after saponification. The identity of major compounds is shown in the chromatogram.

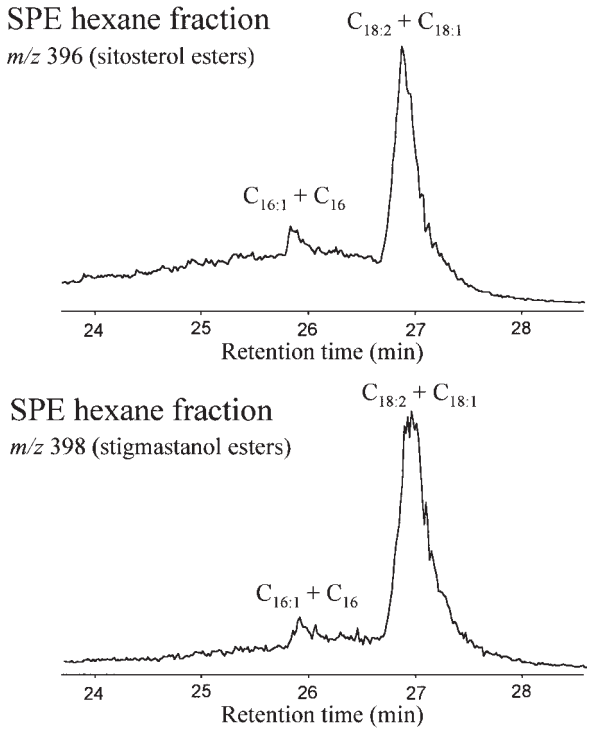

Fig. 3. Mass chromatograms of the ions *m/z* 396 and *m/z* 398 in the SPE hexane fraction showing the distribution of sitosterol and stigmastanol esters, respectively. The identity of the fatty acyl moieties esterified to the different sterols, shown in each peak, has been assumed after the saponification results.

Table 3. Composition (%) of mono-di- and triglycerides

Compounds	%
Monoglycerides	
2-monopalmitin	6
1-monopalmitin	41
2-monostearin	5
1-monostearin	48
Diglycerides	
PM	1
PPo + P2	24
PO + PoSt	63
O2 + Ost	12
Triglycerides	
MP2	3
P3	36
P2O + P2St	61

M: myristic acid, P: palmitic acid, Po: palmitoleic acid, St: stearic acid, O: oleic acid.

compounds responsible for pitch deposition. Among these solutions, the biological elimination of wood extractives prior to pulping represents a promising approach to control pitch problems in pulp and paper manufacture.

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References

Affleck, R.R. and R.G. Ryan. 1969. Pitch control in a kraft pulp mill. Pulp Paper Mag. Can. *70* (12), T563–T567.

Allen, L.H. 1988. Pitch control during the production of aspen kraft pulp. Pulp Paper Can. *89* (10), 87–91.

Allen, L.H., B.B. Sitholé, J.M. MacLeod, C.L. Lapointe and F.J. McPhee. 1991. The importance of seasoning and barking in the kraft pulping of aspen. J. Pulp Paper Sci. *17* (3), J85–J91.

Charrier, B., M. Marques and J.P. Haluk. 1992. HPLC analysis of gallic and ellagic acids in European Oakwood (*Quercus robur* L.) and Eucalyptus (*Eucalyptus globulus*). Holzforschung *46*, 87–89.

Chen, T., Z. Wang, Y. Zhou, C. Breuil, O.K. Aschim, E. Yee and L. Nadeau. 1995. Using solid-phase extraction to assess why aspen causes more pitch problems than softwoods in kraft pulping. Tappi J. *78* (10), 143–149.

Christie, W.W. 1982. Lipid analysis, 2nd Edition. Pergamon Press, Oxford, pp. 51–61.

Conde, E., E. Cadahía, M.C. García-Vallejo and M.B. Fernández de Simón. 1995. Polyphenolic composition of wood extracts from *Eucalyptus camaldulensis*, *E. globulus* and *E. rudis*. Holzforschung *49*, 411–417.

Dunlop-Jones, N., H. Jialing and L.H. Allen. 1991. An analysis of the acetone extractives of the wood and bark from fresh trembling aspen: Implications for deresination and pitch control. J. Pulp Paper Sci. *17* (2), J60–J66.

Evershed, R.P., M.C. Prescott, N. Spooner and L.J. Goad. 1989. Negative ion ammonia chemical ionization and electron impact ionization mass spectrometric analysis of steryl fatty acyl esters. Steroids *53*, 285–309.

Fengel, D. and G. Wegener. 1984. Wood Chemistry, Ultrastructure, Reactions. Walter de Gruyter, Berlin.

Gutiérrez, A., J.C. del Río, F. González-Vila and F. Martín. 1998. Analysis of lipophilic extractives from wood and pitch deposits using solid-phase extraction and gas chromatography. J. Cromatogr. *823*, 449–455.

Hillis, W.E. 1962. Wood Extractives and their Significance to the Pulp and Paper Industry. Academic Press, New York.

Hillis, W.E. and M. Sumimoto. 1989. Effect of extractives on pulping. *In*: Natural Products of Woody Plants II. Ed. J.W. Rowe. Springer-Verlag, Berlin, pp. 880–920.

Leone R. and C. Breuil. 1998. Filamentous fungi can degrade aspen steryl esters and waxes. Int. Biodeter. Biodegr. *41*, 133–137.

Lusby, W. R., M. J. Thompson and J. Kochansky. 1984. Analysis of sterol esters by capillary gas chromatography electron impact and chemical ionization-mass spectrometry. Lipids *19*(11), 888–901.

Mutton, D.B. 1962. Wood resins. *In*: Wood Extractives. Ed. W.E. Hillis. Academic Press, New York, pp. 331-363.

Örså, F. and B. Holmbom. 1994. A convenient method for the determination of wood extractives in papermaking process waters and effluents. J. Pulp Paper Sci. *20*(12), J361–J366.

Rowe, J. W. 1989. Natural Products of Woody Plants II. Springer-Verlag, Berlin.

Sitholé, B. B., J. L. Sullivan and L.H. Allen 1992. Identification and quantitation of acetone extractives of wood and bark by ion

exchange and capillary GC with a spreadsheet program. Holzforschung *46*(5), 409–416.
Sjöström, E. 1993. Wood Chemistry. Fundamentals and Applications, 2nd edition, Academic Press, San Diego, USA.
Swan, B. 1967. Extractives of unbleached and bleached prehydrolysis-kraft pulp from *Eucalyptus globulus*. Svensk Papperstidn. *70*, 616–619.
Swan, B. and I.S. Åkerblom. 1967. Wood extractives from *Eucalyptus globulus* Labill. Svensk Papperstidn. *70*, 239-244.
Wakeham, S.G. and N.M. Frew. 1982. Glass capillary chromatography-electron impact and chemical ionization-mass spectrometry. Lipids *17*(11), 831–843.
Yazaki, Y. and W.E. Hillis. 1976. Polyphenols of *Eucalyptus globulus*, *E. regnans* and *E. deglupta*. Phytochem. *15*, 1180–1182.

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